Determination of Fatty Acid Composition of Amaranthus Species

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Fatty acid compositions of the seed oils from eighteen varieties of amaranthus species have been determined after room temperature transesterification. Consistent with earlier studies, wide variations in the fatty acid composition are reported, and appear to be agronomically related. All varieties show significant levels (2-5%) of squalene and a combined linoleic acid and oleic acid occurrence of between 70-80%. This study represents the first reported fatty acid composition of grain amaranthus cultivated in West Africa.

As part of an effort to introduce grain amaranth into West Africa, eighteen imported amaranthus varieties have been cultivated over two growing seasons at Ile-Ife, Nigeria. Amaranthus species, though native to the tropics (South America, Africa, Asia), are currently cultivated on a commercial level in the midwestern part of the United States. Extensive plant breeding and agronomic studies have been carried out at the Rodale Research Center, Kutztown, Pennsylvania, from where improved lines that are high-yielding in grains and amenable to mechanical cultivation are now available. The milled grains are used to make bread, baked goods and several other cereal-based products (1).

In West Africa, amaranthus species (known as "Efo Tete" in Nigeria) are used solely as ingredients for vegetable soup, while the seeds are discarded; thus the West African varieties are regarded as vegetable types. Extensive chemical analyses have been carried out to determine the nutritional value of several varieties of amaranthus seeds (2,3), most of which are of the grain type cultivated in the United States. Of the many reported studies on amaranthus grain, only the recent study by Saunders and co-workers (3) indicated the possibility of a correlation between lipid composition and agronomic parameters. They reported wide variations in the fatty acid composition of amaranthus seed oils, even between members of the same species.

In this present communication, we report the fatty acid composition of eighteen varieties of amaranthus that were grown under the same agronomic conditions. Fatty acid composition of three lines that were cultivated at two successive growing seasons is also reported. In addition, a modification of an earlier reported seed oil analytical procedure is given.

MATERIALS AND METHODS

Experimental plantings of grain amaranth were initiated in June and September, 1987, at the Agriculture Teaching and Research Farm, Obafemi Awolowo University, Ile-Ife, Nigeria. Grain samples from the June and September plantings were sent to Howard University for analysis. Each study sample was ground into a powder with mortar and pestle, then subjected to a 24 hr Soxhlet extraction with glass distilled pentane as solvent. The extracts, evaporated to afford light golden colored oils (2-5 wt %), were subsequently methylated (described below) for gas chromatographic analysis. A Finnigan gas chromatograph (model 9611) equipped with a splitless injector and interfaced with a Finnigan MAT 4500 atuomated mass spectrometer with a SUPERINCOS data system was used. High resolution capillary gas chromatography was obtained with the use of Supelco fused silica SPB-1 and SPB-5 columns (30 m, 0.32 mm I.D., 0.25 μm film) respectively, temperature programmed from 50°C to 300°C, and helium as carrier gas with a head pressure of 10 psi. The interface oven and transfer line were maintained at 300°C, the ionizer temperature setting was at 140°C, electron energy at 70 eV and injector temperature at 250°C. The mass spectrometer was operated in the electron impact (EI) mode, with an emission current of 0.30 mA, and electron multiplier at 1300 V.

Characterization of the major fatty acid methyl esters was accomplished by comparison of the retention times and mass spectra with those of authentic compounds. Quantitation was achieved by using known quantities of squalane as internal standards with an appropriate response factor for each component methyl ester (4). Response factors were determined from chromatographic peak areas of standard samples.

Preparation of methyl esters. The procedure described here is a slight modification of an earlier transesterification method that we developed to enable rapid screening of a large number of seed oils (4). This modification prevents formation of a cloudy suspension in the analytical sample. The following is a typical analytical procedure: transfer 2-5 mg crude seed oil to a 5-10 ml glass vial, add 800 μl methanol, followed by 200 μl diazomethane-ether solution (4). Shake thoroughly and let stand for one min; add 0.3-0.5 mg squalane as internal standard (the squalane is added from a methylene chlorine solution). Inject 0.2-2.0 μl of sample into the gas chromatograph to quantitate the free fatty acid (FFA). After a minimum of triplicate analyses, add 16 μl 3.3M CH_3ONa/CH_3OH solution. Shake thoroughly for a few seconds and let stand for 5-10 min, then add 10 μl acetic acid. Inject between 0.2-2 μl into the gas chromatograph for analysis of total fatty acid composition. This procedure differs from the previous method only because a solution of methanol-diazomethane-ether was used for the FFA determination. In the latter procedure, a diazomethane-ether solution was used. In this improved procedure, the analytical samples are clear solutions, instead of the cloudy suspensions resulting from the previous method (4).

RESULTS AND DISCUSSIONS

Concerning the slight modification in the transesterification procedure relative to our recent publication (4), it was

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TABLE 1

Weight Percent Oils in Amaranthus Grains and Percent Squalene in the Seed Oils

Species	Breeding line[a]	Trial planting dates	% Oil	% Squalene in oil
A. cruentus	Local[b]	6/4/87	2.49	3.76
A. cruentus	Local[b]	6/4/87	2.42	NA
A. cruentus	1011	6/4/87	2.92	4.05
A. cruentus	1034	6/4/87	2.12	2.24
A. cruentus	434	6/4/87	3.85	NA
A. hypochondriacus	674[b]	6/4/87	1.94	1.88
A. hypochondriacus	718	6/4/87	3.29	3.35
A. hypochondriacus	646	6/4/87	4.08	2.68
A. hypochondriacus	1046	6/4/87	3.18	NA
A. caudatus	713	6/4/87	3.49	3.78
A. caudatus	988	6/4/87	1.70	NA
A. hybridus	1004[b]	6/4/87	2.25	2.40
A. cruentus	1011	9/16/87	3.16	ND
A. cruentus	434	9/16/87	2.77	NA
A. hypochondriacus	1046	9/16/87	1.86	2.29
A. hypochondriacus	674[b]	9/16/87	1.71	2.95
A. hypochondriacus	1023	9/16/87	3.74	2.20
A. hypochondriacus	1024	9/16/87	3.13	2.94
A. hybridus	1047	9/16/87	3.06	4.66

[a]From Rodale Research Center, Kutztown, Pennsylvania.
[b]Dark-seeked variety.
NA = not analyzed; ND = none detected.

TABLE 2

Relative Weight Percent of Fatty Acids in Amaranthus Grain[a]

Species	Breeding line[b]	Trial planting dates	C16:0	C17:0	C18:2	C18:1	C18:0	C20:0
A. cruentus	Local[c]	6/4/87	19.96(0.70)	0.02	52.83(3.17)	24.52(4.07)	2.68	0.01
A. cruentus	Local[c]	6/4/87	19.31(1.00)	ND	52.18(4.01)	25.11(2.21)	3.40	ND
A. cruentus	1011	6/4/87	21.14(1.51)	ND	43.94(3.15)	33.23(2.40)	1.61	ND
A. cruentus	1034	6/4/87	19.39(2.13)	0.11	47.71(1.00)	29.46(0.99)	2.67	0.65
A. cruentus	434	6/4/87	19.07(0.40)	0.08	42.30(1.18)	34.68(0.15)	3.15	0.71
A. hypochondriacus	674[c]	6/4/87	15.06(4.32)	ND	44.67(4.71)	38.72(1.36)	1.36	ND
A. hypochondriacus	718	6/4/87	17.36(1.59)	ND	58.74(1.69)	21.30(0.32)	2.54	0.06
A. hypochondriacus	646	6/4/87	16.96(0.62)	ND	44.68(1.18)	37.20(0.92)	1.07	0.09
A. hypochondriacus	1046	6/4/87	19.52(2.38)	ND	50.44(1.42)	27.43(2.24)	2.62	ND
A. caudatus	713	6/4/87	18.04(0.52)	ND	42.03(0.52)	36.62(2.32)	2.79	0.53
A. caudatus	988	6/4/87	17.65(0.22)	ND	55.97(5.15)	25.87(4.31)	0.57	ND
A. hybridus	1004[c]	6/4/87	19.69(2.14)	ND	54.15(1.65)	23.98(1.11)	2.19	ND
A. cruentus	1011[d]	9/16/87	23.17(2.11)	0.40	37.61(0.86)	34.07(1.86)	4.93	0.10
A. cruentus	434	9/16/87	19.25(1.68)	ND	40.02(2.54)	36.90(2.61)	3.83	ND
A. hypochondriacus	1046[d]	9/16/87	20.34(0.37)	ND	47.97(1.36)	27.01(1.72)	4.68	ND
A. hypochondriacus	674[c]	9/16/87	18.00(2.50)	ND	56.68(2.15)	24.46(1.83)	0.86	ND
A. hypochondriacus	1023	9/16/87	19.55(1.26)	0.14	47.09(0.90)	29.85(1.01)	2.94	0.44
A. hypochondriacus	1024	9/16/87	18.96(1.03)	ND	48.03(1.18)	30.32(1.45)	2.70	ND
A. hydridus	1047	9/16/87	14.32(0.21)	ND	50.72(3.13)	31.02(2.96)	3.91	ND

[a]Minimum of triplicate determination for each sample. Standard deviations given in parentheses (±).
[b]From Rodale Research Center, Kutztown, Pennsylvania.
[c]Dark-seeded variety.
[d]Contain traces of linolenic acid.

TABLE 3

Relative Weight Percent of Fatty Acids from Three Amaranthus Lines Cultivated at Two Successive Growing Seasons

Species	Breeding line	Trial planting dates	C16:0	C17:0	C18:2	C18:1	C18:0	C20:0
A. hypochondriacus	674	6/4/87	15.06	ND	44.67[a]	38.92[b]	1.36[c]	ND
		9/16/87	18.00	ND	56.68[a]	24.46[b]	0.86[c]	ND
A. cruentus	1011	6/4/87	21.24	ND	43.94[d]	33.23	1.61[e]	ND
		9/16/87	23.17	0.04	37.61[d]	34.07	4.93[e]	ND
A. hypochondriacus	1046	6/4/87	19.52	ND	50.44[f]	27.43	2.62	ND
		9/16/87	20.34	ND	47.97[f]	27.01	4.68	ND

Values with same superscript are significant at $P < 0.05$. ND = none detected.

initially made necessary in order to prevent the occurrence of persistent but minor amounts of fatty acid esters with a mass spectral base peak at m/z 88, for saturated esters, and for esters with odd-numbered carbon atoms. We attributed these to the formation of α-methyl esters. However, upon closer scrutiny, and reference to some existing literary evidence (5), these minor components are now believed to be ethyl esters, the origin of which still remain unknown.

The percentage of oil in amaranthus grain and the percentage of squalene in the seed oils are given in Table 1. Analyses of the oils indicate insignificant levels of free fatty acids in most of the varieties, suggesting the absence of lipase activity in amaranthus seed. Table 2 gives the relative percentage of the component fatty acids in the oils indicating a pronunced variation, both interspecific and intra-specific. Based on our data, it is safe to conclude that there is no typical amaranthus seed oil. The variation in the fatty acid composition appears to be agronomically related ($P < 0.05$). However, these variations can also be due to genetic factors, inherent in the individual plant. Support for the agronomic variation is derived from the fatty acid composition from one generation to another (Table 3). The agronomic parameters for this project indicate little rainfall for the September planting (Oke, O. L., G. Adegoroye and Y. Akinyemiju, Awolowo University, Ile-Ife, Nigeria, unpublished work). Another conclusion worthy of note is that the percentage of unsaturated fatty acid in amaranthus seed oil is generally between 70–80%, while only traces of linolenic acid were detected in a few varieties. Thus amaranthus grain is a source of nutritionally important fatty acids, especially linoleic acid, the most prominent component found in all species.

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